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Analytical Methods

Determination of biogenic amines in foods using ultra-performance liquid chromatography (UPLC)

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ABSTRACT

A rapid ultra-performance liquid chromatographic (UPLC) method for the determination of biogenic amines (putrescine, cadaverine, spermidine, spermine, phenylethylamine, histamine, tyramine and tryptamine) in selected food samples is described. The eight biogenic amines, which are the most important to be determined in food samples, were derivatized with dansyl chloride prior to UPLC separation. The dansylated amines were separated on an Agilent Zorbax Eclipse XDB – C18 column (50 × 4.6 mm ID, 1.8 μm) using gradient elution with a binary system of acetonitrile–water, a flow rate of 1.0 ml/min and UV detection at 225 nm. The analysis is very fast, all amines are well resolved and are eluted from the column in less than 6 min. The average repeatability of the method ranged between 1.02% and 2.14%. Limits of detection (LODs) for considered amines ranged between 0.032 and 0.098 μg/l; calibration curves showed very good linearity (r = 0.9994–1.0000). The method was applied to the analysis of amines in pork, beef, chicken and fish meat, cheese and edible mushrooms.

1. Introduction

Biogenic amines (BAs), such as putrescine (PUT), cadaverine (CAD), spermidine (SPD), spermine (SPM), tryptamine (TRM), phenylethylamine (PEA), histamine (HIM) and tyramine (TYM) are low molecular weight compounds arising via several biochemical pathways (Miyazaki & Yang, 1987). Small amounts are biosynthesised in plant and animal cells but they are also produced in foods in larger quantities by microbial decarboxylation of amino acids. *Enterobacteriaceae* rank among the microorganisms primarily responsible for decomposition of scombroid fish and the most potent histamine producers (Baixas-Nogueras, Bover-Cid, Veciana-Nogues, Marine-Font, & Vidal-Carou, 2005). *Morganella morganii*, *Klebsiella pneumoniae*, *Hafnia alvei* were found in tuna fish abused by elevated temperature (Lehane, 2000). BAs are normal constituents of fermented foods such as cheese, wine, beer, sauerkraut etc. The amount and type of amine formed is influenced by the food composition, microbial flora and by several parameters, which promote bacterial growth during storage – such as temperature, ripening and packaging (Halasz, Barath, Simon-Sarkadi, & Holzapfel, 1994; Křížek, Vácha, Vorlová, Lukášová, & Cupáková, 2004). Food quality has been linked to these amines in fermented but, primarily in non-fermented foodstuffs (Ozogul, Ozogul, & Kuley, 2008). There are two reasons for the determination of amines in foods: the first is their potential toxicity; the second is the possibility of using them as food quality markers. Amine toxicity depends strongly on the individual efficiency of detoxication (Shalaby, 1996). During the food intake process in the human gut, low amounts of BAs are metabolised to physiologically less active degradation products. This detoxification system includes specific enzymes such as diamine oxidases (DAO). However, when large amounts of BAs are ingested, the detoxification system is unable to eliminate them sufficiently. Genetic predisposition, gastrointestinal disease or inhibition of DAO activity due to medicines or alcohol may result in suppressed detoxication of amines (Křížek, Pavlíček, & Vácha, 2002). For sensitive individuals they represent a health risk, especially when their effect is potentiated by other substances.

Most toxic are HIM and TYM. HIM poisoning is mainly related to the consumption of fish belonging to the *Scombridae* family. Many outbreaks of HIM poisoning are periodically reported from all over the world (Chen et al., 2008). HIM poisoning has a short incubation period, ranging from minutes to a few hours after ingestion. Symptoms include headache, facial flushing and sweating, rash and itching, nausea, vomiting, diarrhea and heart palpitations (Becker, Southwick, Reardon, Berg, & MacCormack, 2001). Ingestion of food rich in TYM may result in a dangerous intoxication, known as the *cheese reaction*: a hypertensive crisis, usually accompanied by a severe headache. This sometimes led to intracranial haemorrhage, neuronal sequelae, cardiac failure and pulmonary edema (Joosten, 1988). Other BAs, e.g. PUT and CAD potentiate the toxicity of HIM

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and TYM. Good standards of hygiene in handling with foodstuffs together with relevant storage temperature or modified atmosphere packaging (Balamatsia, Patsias, Kontominas, & Savvaidis, 2007) play the key roles in preventing the formation of BAs.

Methods for the determination of BAs in foods are numerous. The first procedures developed focused on the most toxicologically important amines – HIM and TYM. For the determination of amines thin layer chromatography (TLC), ion exchange chromatography (IEC), gas chromatography (GC) and especially high performance liquid chromatography (HPLC) have been proposed. Good results are also provided by capillary zone electrophoresis (CZE).

As amines are strong organic bases it is very useful to take advantage of this feature for their separation from sample matrix. For this purpose perchloric acid is widely used as extraction agent. This important preliminary step was discussed in detail by Moret and Conte (1996). Amines are solely separated as derivatives when using liquid chromatographic techniques. Dansyl chloride has been the most widely employed reagent for derivatization of amines. TLC of dansylated amines has continued to be used due to its quickness, simplicity and the possibility of the screening of several samples at a time. Therefore, the TLC method can be effectively used, e.g. in the food industry (Shakila, Vasundhara, & Kumudavally, 2001). A wide variety of organic solvents was proposed for TLC separations, modern methods prefer non toxic ones. The detection limits are 5–10 ng on the spot when applying the fluorescence densitometry (Lapa-Guimaraes & Pickova, 2004).

Ion chromatography is suitable especially for complex matrices. Perchloric acid extracts were injected without any further treatment onto cation exchange column and were eluted by methanesulfonic acid solution. The limits of detection 7–12 mg/kg were higher compared to other chromatographic techniques (Favaro, Pastore, Saccani, & Cavalli, 2007). The possibility of employing an amino acid analyser is a big advantage of IEC.

Gas chromatography is not so often applied for the determination of amines. Because of inherent tailing problems, derivatization is also frequently used. In beers and wines a two-phase *O*-ethoxycarbonylation was performed with ethyl chloroformate to alkylphenols with subsequent *N*-ethoxycarbonylation of amines (Paik, Choi, & Kim, 2006). For amines in alcoholic beverages a derivatization procedure with isobutyl chloroformate was proposed (Fernandes, Judas, Oliveira, Ferreira, & Ferreira, 2001). Both methods were designed for mass spectrometry detection. BAs in fish samples were derivatized with pentafluoropropionic anhydride. Separations were achieved using a capillary DB-1 column connected to FID detector (Du et al., 2002).

Aromatic and heterocyclic BAs can be easily determined by capillary zone electrophoresis (CZE) without derivatization due to their inherent absorption of UV light. The analyses of HIM are fast (migration times are within 4–9 min), run in plain fused silica capillaries in simple buffer solutions. HIM in fish was determined in phosphate buffer, pH 2.5 (Rossano, Mastrangelo, Ungaro, & Riccio, 2006). For tomatoes or tomato paste samples a citrate buffer, pH 2.5 was used (Bolygo, Cooper, Jessop, & Moffatt, 2000). Generally, amines must be derivatized in CZE. For food samples 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate was proposed. Seven BAs – PUT, CAD, SPD, SPM, HIM, TYM and TRM, most commonly found in foods, were resolved within 25 min (Kovacs, Simon-Sarkadi, & Ganzler, 1999). The same group of amines was resolved within 15–35 min using a very simple derivatization with benzoylchloride (Křížek & Pelikánová, 1998). Derivatization with 4-fluor-7-nitro-2,1,3-benzoxadiazole and fluorescence detection meets the requirements for very sensitive determination of amines in plant tissues (Zhang, Tang, & Sun, 2005). HPLC procedures involve pre- or post-column derivatization step. BAs can be separated as ion pairs on reversed-phase HPLC. Sodium octanesulfonate has been often used as ion pairing agent, amines are derivatized in a post-column mode by *o*-phthalaldehyde (Santos, Souza, Cerqueira, & Gloria, 2003). Many reagents were proposed for derivatization of BAs prior to HPLC. Vandenabeele et al. used 2-chloroethylnitrosourea (Vandenabeele, Garrelly, Ghelfenstein, Commeyras, & Mion, 1998), but most frequently used is probably dansyl chloride (Hernandez-Borges, D'Orazio, Aturki & Fanali, 2007; Proestos, Loukatos, & Komaitis, 2008; Soufleros, Bouloumpasi, Zotou, & Loukou, 2007).

Since 2004 a new generation of stationary phases which withstand very high pressures (up to 1000 bar) as well as compatible LC systems have been commercialised from several suppliers under the trade name Ultra Performance Liquid Chromatography (UPLC) (Nguyen et al., 2007). UPLC, using small particles (<2 μm) in short columns (5 cm) can drastically decrease the analysis time without loss in efficiency. Comparing to the conventional HPLC method, UPLC showed many advantages, including reduced run time, less solvent consumption and increased peak capacities (Liu et al., 2007).

The method developed in our laboratory is based on UPLC separation of dansylamides. The method was applied to selected food samples.

2. Experimental

2.1. Reagents

The mixed standard solution of BAs was prepared from the following reagents: Putrescine dihydrochloride 99% (purity), cadaverine dihydrochloride 99%, histamine dihydrochloride 99%, tyramine hydrochloride 97%, tryptamine hydrochloride 98%, 1,7-heptanediamine (HEP) 97% (Fluka, Buchs, Switzerland); spermidine trihydrochloride 99.5%, spermine tetrahydrochloride 99.5%, phenetylamine hydrochloride 99%, dansyl chloride 95% (Dns-Cl) (Sigma–Aldrich, St.Louis, MO, USA). Other chemicals: proline, heptane (Fluka, Buchs, Switzerland); acetonitrile (Merck, Darmstadt, Germany); Na_2CO_3, K_2CO_3, acetone (Penta, Chrudim, Czech Republic); $NaHCO_3$ (Lach-Ner, Neratovice, Czech Republic); perchloric acid (Acros, Geel, Belgium). All chemicals were of analytical grade or higher. Deionized water was prepared with Premier equipment (Premier systems, Phoenix, AZ, USA). Standard (stock) solution of BAs was prepared at a concentration around 400 mg/l in 0.6 M $HClO_4$ and was further diluted for experiments.

2.2. Apparatus

Samples were homogenised with either commercial food handblender (Philips) or Ultra-Turrax T25 homogeniser (Ika Labortechnik, Staufen, Germany). Other instruments: Sigma 2-5 centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany); Thermovap (Ecom s.r.o., Prague, Czech Republic) for heptane extracts evaporation; vacuum filtration assembly Swinnex (Millipore, Carrigtwohill, Ireland); glass microfibre filters (12 mm diameter and 1.7 μm pore size) (Filpap, Štětí, Czech Republic).

UPLC was carried out on Agilent 1200 Series Rapid Resolution LC system (Agilent Technologies, Inc., Santa Clara, CA, USA). The system was equipped with binary pumps, a micro-vacuum degasser, high performance autosampler and diode array detector. Data processing was performed using ChemStation for LC 3D systems (Agilent Technologies).

2.3. Sample preparation

Food sample (40 g) was homogenised in a plastic beaker with 100 ml of 0.6 M $HClO_4$ for 3 min. The slurry was centrifuged for 10 min at 1800 *g* (3500 rpm). The supernatant was then filtered through a filter paper, washed with $HClO_4$ and the volume was

recorded. One millitre of acidic extract was spiked with 100 μl of internal standard solution (1,7-heptanediamine, 400 mg/l) and mixed in a plastic test tube with 1.5 ml of carbonate buffer pH 11 (Section 2.3.1). After briefly vortexing, 2 ml of dansyl chloride solution were added (dansyl chloride in acetone 5 mg/ml). The test tube was then shaken at room temperature for 20 h in darkness. Subsequently 200 μl of proline solution (100 mg/ml) were added and the sample was shaken for additional 1 h. Then follows an extraction with 3 ml of heptane. One millilitre of extract was dried at 60 °C under a stream of nitrogen. The dry residue was dissolved in 1.5 ml of acetonitrile. Samples were filtered through membrane glass fiber filters (1.7 μm) prior to analysis.

2.3.1. Preparation of carbonate buffer

Fifty millilitre of solution A (0.5 M $NaHCO_3$) were mixed with 12 ml of solution B (0.5 M Na_2CO_3). The pH was then adjusted to 9.2 by adding small volume (ca. 0.5 ml) of the solution B. This buffer mixture was stable for several months in refrigerator.

Prior to derivatization, 16.65 g of K_2CO_3 were dissolved in 50 ml of previously prepared buffer (pH 9.2). The pH value is thereby increased to 11.0–11.1. This carbonate buffer for derivatization must be freshly prepared.

2.4. Separation conditions

The UPLC separation was carried out on an Agilent Zorbax Eclipse XDB – C18 column (50 mm × 4.6 mm ID, 1.8 μm particle size), equipped with a RRLC In-line filter, 0.2 μm (Agilent Technologies, Inc., Santa Clara, CA, USA). Chromatographic separation was carried out using a gradient elution of (A) acetonitrile (100%), (B) acetonitrile (50%) as follows: 0–2 min, A 40%, B 60%, 2–3 min, A 40–80%, B 60–20%, 3–4 min, A 80–90%, B 20–10%, 4–6 min, A 90–95%, B 10–5%, 6–7 min, A 95–40%, B 5–60%, 7–12 min, A 40%, B 60%. Linear concentration change was performed in all cases. The flow rate was kept at 1.0 ml/min, column temperature at 25 °C, injection 5 μl, the detection wavelength at 225 nm.

3. Results and discussion

The apparent difference between ultra-performance liquid chromatography (sometimes also called Rapid resolution liquid chromatography) and high performance liquid chromatography is mainly in the particle size. It is not only the increased surface area at the external surfaces of small-sized particles, but the faster equilibration of total surfaces including those inside the mesopores and the smaller flow irregularity based on the smaller particle size that provide higher column efficiency per unit time than a conventional column packed with larger-sized particles. Ultra-performance liquid chromatography brings new quality to the separation process.

3.1. Extraction from sample matrix

Extraction of amines from food samples is an important step prior to the separation. Most of the methods available in the literature for biogenic amine determination involve an acidic extraction from a solid matrix, organic solvents are seldom used. The choice of acid has to be related to the characteristics of the matrix to be analysed. Many compounds that might interfere can be eliminated in this step. Hydrochloric acid appears to be a good choice for the analysis of cheese, however, it is not suitable acid for fish or meat products due to the difficulties related to occasional sample turbidity. In the case of meat samples trichloroacetic acid is a better choice because of its capacity to precipitate proteins (Moret & Conte, 1996). According to our experience, perchloric acid represents an acceptable compromise to wide variety of food samples (Křížek, 1991; Křížek & Pelikánová, 1998).

3.2. Derivatization

An optimisation of the derivatization procedure was carried out, by investigating the influence of derivatization temperature, derivatization time and Dns-Cl concentration on the output of the dansylation reaction. Derivatization at elevated temperature is used by some authors, because high temperature promotes the derivatization reaction and the process is completed in short time. Temperature 40 °C for 45–60 min has been most frequently used (Dugo, Vilasi, La Torre, & Pellicano, 2006; Proestos et al., 2008). Shakila et al.(2001) uses 55 °C for 1 h, Hernandez-Borges, D'Orazio, Aturki and Fanali (2007) uses 65 °C for 25 min. The temperatures higher than 65 °C may cause the decomposition of dansylamides resulting in deterioration of peak shape and the decreased peak areas (Zotou, Loukou, Soufleros, & Stratis, 2003). Summarizing briefly, the higher temperature for dansylation, the shorter time for derivatization. The derivatization of standard solutions both at 20 °C (room temperature) for 20 h and at 50 °C for 1 h was compared. The mean between-day precision for all eight amines in 10 samples was RSD = 1.50% (at 20 °C; 20 h) and RSD = 2.55% (for 50 °C; 1 h). Though temperatures up to 65 °C were acceptable, lower temperature for longer time was preferred. In case of a good laboratory work organisation (derivatization overnight), the sample preparation proceeds fluently. Since derivatives are sensitive to light, the reaction should be done in darkness. It is also possible to use alkaline buffer pH 11.0 when derivatization proceeds at room temperature. The efficiency of the reaction is thus higher and the risk of decomposition of derivatives is low at 20 °C.

The amount of Dns-Cl used in the derivatization was proved by Zotou et al. (2003) who added 4–12 mg of Dns-Cl to 1–2 ml of the buffered sample extract. They considered the amount of 8 mg of Dns-Cl to be optimum for the reaction. Our experiments with samples containing similar amounts of amines showed, that 2 ml of Dns-Cl solution (2.5 mg/ml in acetone) were acceptable for all amines but not for SPM, its calibration curve was not linear. When the amount of Dns-Cl was increased, addition of 2 ml (5 mg/ml in acetone), all amines showed linear calibration curves. The relative amount of Dns-Cl was in good accordance with Zotou et al. (2003).

3.3. Chromatographic conditions

Fig. 1 shows the chromatogram of a standard solution of dansylamides, obtained with the gradient profile described in Section 2.4. The gradient profile was carefully designed. The content of acetonitrile in the mobile phase can be increased after 2 min from the sample injection. Earlier increase of acetonitrile content leads to poor resolution of CAD and HIM. The content of acetonitrile is maximal in the sixth minute, just after elution of SPM. The high content of acetonitrile in the mobile phase within the fourth and the sixth minute is a necessity because of slow movement of SPD and especially SPM in the column. After SPM elution the composition of the mobile phase returns within 6 min to the initial composition. Thus the complete cycle – the time between consecutive injections – lasts 12 min. Figs. 2 and 3 are examples of analyses of food samples, chicken and fish meat respectively. Sample of chicken meat was taken from chicken leg 30 min after slaughter. Sample of European catfish (*Silurus glanis*) was taken from fish fillets 2 h after gutting. Concentrations of PUT and CAD above 10–20 mg/kg reveal just the moment of the onset of decomposition in these samples (Chytiri, Paleologos, Savvaidis, & Kontominas, 2004; Křížek et al., 2002; Křížek et al., 2004). Contents of HIM and TYM found in theses samples are negligible from toxicological point of view. Both food samples are thus of good hygienical quality.

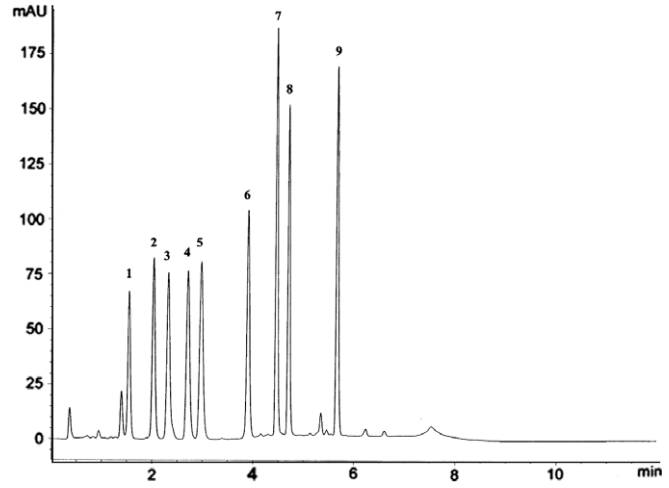

Fig. 1. Separation of standard mixture of biogenic amines as dansylamides. Peaks numbering: 1 – TRM, 2 – PEA, 3 – PUT, 4 – CAD, 5 – HIM, 6 – HEP (I.S.), 7 – TYM, 8 – SPD, 9 – SPM. Conditions as in Section 2.4. Concentrations of the amines are in the acetonitrile solution for injection: TRM – 8.96 mg/l, PEA – 9.08 mg/l, PUT – 8.90 mg/l, CAD – 9.04 mg/l, HIM – 9.56 mg/l, HEP – 9.12 mg/l, TYM – 9.19 mg/l, SPD – 8.95 mg/l, SPM – 9.21 mg/l.

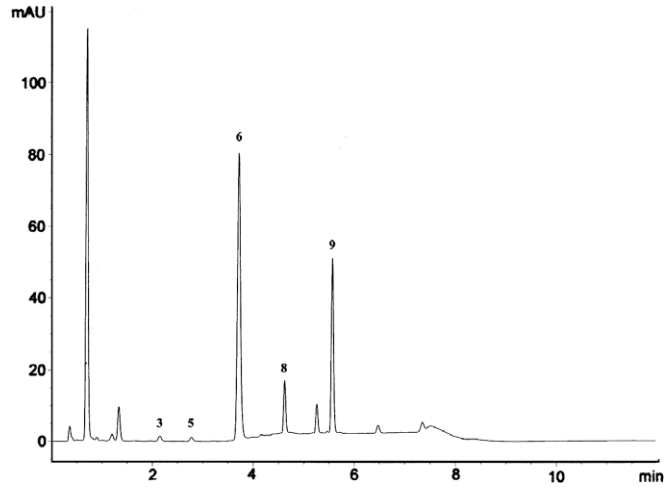

Fig. 2. Analysis of chicken meat. Conditions as in Section 2.4. Content of amines in the sample: PUT – 2.19 mg/kg, HIM – 2.28 mg/kg, SPD – 11.1 mg/kg, SPM – 27.3 mg/kg. Numbers as in Fig. 1.

3.4. Chromatographic and method parameters

Amines were identified on the basis of retention time by comparison with standard solutions. Retention times of the amines were stable and consistently reproducible. Major side-products of the dansyl reaction were eluted by 30–60 s and good separation was achieved from derivatized amines. Dansylated amines are completely resolved and eluted from the column within 4–5 min (Fig. 1). Selected chromatographic parameters for UPLC column are given in Table 1. Separation is much faster and more effective than separation on standard HPLC columns with elution time range of 20–30 min. Data given in Table 1 reveal very high efficiency of UPLC system. Parameters N/l and H are four times, N_{ef}/t_r 15 times better for UPLC compared to standard HPLC (compared in the laboratory of authors on Phenomenex column, 150 mm × 2.0 mm ID, 3 μm particle size). The proposed concentration range of the method (2–150 mg/kg) is sufficient for most food samples. Lower contents of amines are not toxicologically important. Higher values are sometimes observed in spoiled fish samples, acidic extracts of

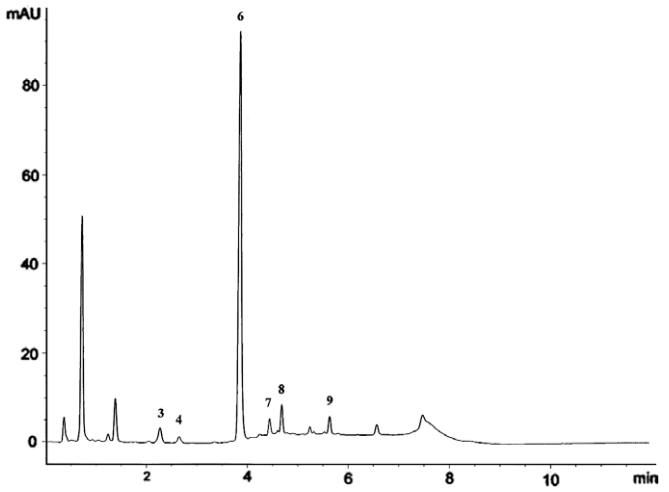

Fig. 3. Analysis of fish meat (European catfish, *Silurus glanis*). Conditions as in Section 2.4. Content of amines in the sample: PUT – 7.44 mg/kg, CAD – 3.38, TYM – 8.29, SPD – 10.9 mg/kg, SPM – 6.85 mg/kg. Numbers as inFig. 1.

Table 1
Average values ($n = 5$) of selected parameters.

Amine	t_r (min)	N/l (m^{-1})	H (μm)	N_{ef}/t_r (min^{-1})
TRM	1.64	2,16,000	4.62	4000
PEA	2.17	3,47,000	2.88	5540
PUT	2.50	3,64,000	2.75	5320
CAD	2.93	4,41,000	2.26	5780
HIM	3.20	4,69,000	2.13	5750
HEP	4.03	16,50,000	0.607	16,900
TYM	4.53	24,80,000	0.404	23,100
SPD	4.76	28,00,000	0.357	25,100
SPM	5.71	35,40,000	0.283	27,200

t_r – Retention time; N/l – number of theoretical plates per metre; H – plate height; N_{ef}/t_r – number of effective plates per unit of retention time. Unretained marker: acetonitrile 80%; $t_r = 0.36$ min.

such samples can be easily diluted. The values for residuals of standard deviation for linear ($sy1$) and non-linear ($sy2$) calibration function were calculated (1994). According to the results of F-test, all amines have strongly linear responses (relative peak area versus concentration) in the concentration range. Table 2 reports the correlation coefficients and the limit of detection (LOD) and the limit of quantification (LOQ) values. LOD is given as the concentration of the analyte that gives a signal equal to the average background (S_{blank}) plus three times the standard deviation s_{blank} of the blank (LOD = $S_{blank} + 3s_{blank}$), while LOQ is given as LOQ = $S_{blank} + 10s_{blank}$) (Miller & Miller, 2000). All limits are calculated using standard additions to the real sample in order to take into account the possible matrix interference. The repeatability (within-day precision) of the method was assessed by measuring of 10 independent

Table 2
Linearity, LOD and LOQ values calculated by standard addition method in sample of Edam cheese ($n = 10$).

Amine	r	LOD (μg/l)	LOQ (μg/l)	LOD_s (mg/kg)	LOQ_s (mg/kg)
TRM	1.0000	0.078	0.26	0.88	2.9
PEA	0.9998	0.066	0.22	0.74	2.5
PUT	0.9998	0.056	0.19	0.63	2.1
CAD	0.9999	0.052	0.17	0.58	1.9
HIM	0.9999	0.050	0.17	0.56	1.9
TYM	0.9994	0.087	0.29	0.98	3.3
SPD	0.9999	0.032	0.11	0.36	1.2
SPM	0.9997	0.098	0.32	1.12	3.7

LOD_s and LOQ_s are values recalculated for the food sample.

Table 3
The repeatability (A) ($n = 10$) and reproducibility (B) ($n = 5$) of the method given as RSD (%).

Amine	A	B
TRM	1.62	3.37
PEA	1.66	3.81
PUT	1.25	3.08
CAD	1.02	2.77
HIM	1.22	2.95
TYM	1.59	4.27
SPD	1.50	4.21
SPM	2.14	4.66

samples of fish flesh spiked with BAs (corresponding to 50 mg/kg of each amine in flesh). The reproducibility (between-day precision) was calculated from analyses of five independent fish samples prepared and measured in five different days. The results are summarised in Table 3. As can be seen, the relative standard deviations for the within-day precision range from 1.02% to 2.14% and for the between-day precision from 2.77% to 4.66%, indicating a good standard of precision. The method was successfully applied to determine the content of BAs in pork, beef, poultry and fish meat, in various kinds of cheese (mainly Edam type), and edible mushrooms (champignon, boletus) (data not published yet). Other biogenic amines (e.g. agmatine) might be determined by this method. The reaction of agmatine with dansyl chloride is pH dependent (Slocum, Flores, Galston, & Weinstein, 1989). Thus the possibilities of agmatine derivatization in above described buffer system should be examined.

4. Conclusions

The method described in this paper is suitable for the determination of eight biogenic amines in foods using a new UPLC chromatographic technique. The peak resolution is very good and no interferences in real samples were observed. The dansylation reaction was carried out at room temperature. This resulted in the necessity of longer reaction time, but higher derivatization efficiency in more alkaline buffer is likely to occur. Since the chromatographic separation is very fast, the overnight run can handle about eighty samples. Though the working range of method was designed for food samples, lower detection limits required for other matrices (e.g. plant extracts) could be reached by using fluorimetric detection.

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References

(1994). Water quality-calibration and evaluation of analytical methods and estimation of performance characteristics. Part 1: Statistical evaluation of the linear calibration function. In C.S. Institute, CSN-ISO 8466-1, vol. CSN-ISO 8466-1. Prague.

Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogues, M. T., Marine-Font, A., & Vidal-Carou, M. C. (2005). Biogenic amine index for freshness evaluation in iced Mediterranean hake (*Merluccius merluccius*). *Journal of Food Protection, 68*(11), 2433–2438.

Balamatsia, C. C., Patsias, A., Kontominas, M. G., & Savvaidis, I. N. (2007). Possible role of volatile amines as quality-indicating metabolites in modified atmosphere-packaged chicken fillets: Correlation with microbiological and sensory attributes. *Food Chemistry, 104*(4), 1622–1628.

Becker, K., Southwick, K., Reardon, J., Berg, R., & MacCormack, J. N. (2001). Histamine poisoning associated with eating tuna burgers. *Jama – Journal of the American Medical Association, 285*(10), 1327–1330.

Bolygo, E., Cooper, P. A., Jessop, K. M., & Moffatt, F. (2000). Determination of histamine in tomatoes by capillary electrophoresis. *Journal of AOAC International, 83*(1), 89–94.

Chen, H. C., Kung, H. F., Chen, W. C., Lin, W. F., Hwang, D. F., Lee, Y. C., et al. (2008). Determination of histamine and histamine-forming bacteria in tuna dumpling implicated in a food-borne poisoning. *Food Chemistry, 106*(2), 612–618.

Chytiri, S., Paleologos, E., Savvaidis, I., & Kontominas, M. G. (2004). Relation of biogenic amines with microbial and sensory changes of whole and filleted freshwater rainbow trout (*Onchorynchus mykiss*) stored on ice. *Journal of Food Protection, 67*(5), 960–965.

Du, W. X., Lin, C. M., Phu, A. T., Cornell, J. A., Marshall, M. R., & Wei, C. I. (2002). Development of biogenic amines in yellowfin tuna (*Thunnus albacares*): Effect of storage and correlation with decarboxylase-positive bacterial flora. *Journal of Food Science, 67*(1), 292–301.

Dugo, G., Vilasi, F., La Torre, G. L., & Pellicano, T. M. (2006). Reverse phase HPLC/DAD determination of biogenic amines as dansyl derivatives in experimental red wines. *Food Chemistry, 95*(4), 672–676.

Favaro, G., Pastore, P., Saccani, G., & Cavalli, S. (2007). Determination of biogenic amines in fresh and processed meat by ion chromatography and integrated pulsed amperometric detection on Au electrode. *Food Chemistry, 105*(4), 1652–1658.

Fernandes, J. O., Judas, I. C., Oliveira, M. B., Ferreira, I., & Ferreira, M. A. (2001). A GC–MS method for quantitation of histamine and other biogenic amines in beer. *Chromatographia, 53*, S327–S331.

Halasz, A., Barath, A., Simon-Sarkadi, L., & Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in foods. *Trends in Food Science and Technology, 5*(2), 42–49.

Hernandez-Borges, J., D'Orazio, G., Aturki, Z., & Fanali, S. (2007). Nano-liquid chromatography analysis of dansylated biogenic amines in wines. *Journal of Chromatography A, 1147*(2), 192–199.

Joosten, H. M. L. J. (1988). The biogenic amine contents of Dutch cheese and their toxicological significance. *Netherlands Milk and Dairy Journal, 42*(1), 25–42.

Kovacs, A., Simon-Sarkadi, L., & Ganzler, K. (1999). Determination of biogenic amines by capillary electrophoresis. *Journal of Chromatography A, 836*(2), 305–313.

Křížek, M. (1991). The determination of biogenic amines in silage. *Archives für Animal Nutrition, 41*(1), 97–104.

Křížek, M., Pavlíček, T., & Vácha, F. (2002). Formation of selected biogenic amines in carp meat. *Journal of the Science of Food and Agriculture, 82*(9), 1088–1093.

Křížek, M., & Pelikánová, T. (1998). Determination of seven biogenic amines in foods by micellar electrokinetic capillary chromatography. *Journal of Chromatography A, 815*(2), 243–250.

Křížek, M., Vácha, F., Vorlová, L., Lukášová, J., & Cupáková, S. (2004). Biogenic amines in vacuum-packed and non-vacuum-packed flesh of carp (*Cyprinus carpio*) stored at different temperatures. *Food Chemistry, 88*(2), 185–191.

Lapa-Guimaraes, J., & Pickova, J. (2004). New solvent systems for thin-layer chromatographic determination of nine biogenic amines in fish and squid. *Journal of Chromatography A, 1045*(1–2), 223–232.

Lehane, L. (2000). Update on histamine fish poisoning. *Medical Journal of Australia, 173*(3), 149–152.

Liu, M., Li, Y. G., Chou, G. X., Cheng, X. M., Zhang, M., & Wang, Z. T. (2007). Extraction and ultra-performance liquid chromatography of hydrophilic an lipophilic bioactive components in a Chinese herb Radix Salviae Miltiorrhizae. *Journal of Chromatography A, 1157*(1–2), 51–55.

Miller, J. N., & Miller, J. C. (2000). *Statistics and chemometrics for analytical chemistry*. Upper Saddle River, NJ, USA: Prentice Hall.

Miyazaki, J. H., & Yang, S. F. (1987). The methionine salvage pathway in relation to ethylene and polyamine biosynthesis. *Physiologia Plantarum, 69*(2), 366–370.

Moret, S., & Conte, L. S. (1996). High-performance liquid chromatographic evaluation of biogenic amines in foods – An analysis of different methods of sample preparation in relation to food characteristics. *Journal of Chromatography A, 729*(1–2), 363–369.

Nguyen, D. T. T., Guillarme, D., Heinisch, S., Barrioulet, M. P., Rocca, J. L., Rudaz, S., et al. (2007). High throughput liquid chromatography with sub-2 mu m particles at high pressure and high temperature. *Journal of Chromatography A, 1167*(1), 76–84.

Ozogul, F., Ozogul, Y., & Kuley, E. (2008). Nucleotide degradation and biogenic amine formation of wild white grouper (*Epinephelus aeneus*) stored in ice and at chill temperature (4 degree C). *Food Chemistry, 108*(3), 933–941.

Paik, M. J., Choi, Y. M., & Kim, K. R. (2006). Simultaneous profiling analysis of alkylphenols and amines by gas chromatography and gas chromatography–mass spectrometry. *Analytica Chimica Acta, 560*(1–2), 218–226.

Proestos, C., Loukatos, P., & Komaitis, M. (2008). Determination of biogenic amines in wines by HPLC with precolumn dansylation and fluorimetric detection. *Food Chemistry, 106*(3), 1218–1224.

Rossano, R., Mastrangelo, L., Ungaro, N., & Riccio, P. (2006). Influence of storage temperature, freezing time on histamine level in the European anchovy *Engraulis encrasicholus* (L., 1758): A study by capillary electrophoresis. *Journal of Chromatography B – Analytical Technologies in the Biomedical and Life Sciences, 830*(1), 161–164.

Santos, W. C., Souza, M. R., Cerqueira, M., & Gloria, M. B. A. (2003). Bioactive amines formation in milk by Lactococcus in the presence or not of rennet and NaCl at 20 and 32 degrees C. *Food Chemistry, 81*(4), 595–606.

Shakila, R. J., Vasundhara, T. S., & Kumudavally, K. V. (2001). A comparison of the TLC-densitometry and HPLC method for the determination of biogenic amines in fish and fishery products. *Food Chemistry, 75*(2), 255–259.

Shalaby, A. R. (1996). Significance of biogenic amines to food safety and human health. *Food Research International, 29*(7), 675–690.

Slocum, R. D., Flores, H. E., Galston, A. W., & Weinstein, L. H. (1989). Improved method for HPLC analysis of polyamines, agmatine and aromatic monoamines in plant tissue. *Plant Physiology, 89*(2), 512–517.

Soufleros, E. H., Bouloumpasi, E., Zotou, A., & Loukou, Z. (2007). Determination of biogenic amines in Greek wines by HPLC and ultraviolet detection after dansylation and examination of factors affecting their presence and concentration. *Food Chemistry, 101*(2), 704–716.

Vandenabeele, O., Garrelly, L., Ghelfenstein, M., Commeyras, A., & Mion, L. (1998). Use of 2-chloroethylnitrosourea, a new type of pre-column derivatizing agent for the measurement of biogenic amines, by high-performance liquid chromatography with ultraviolet detection. *Journal of Chromatography A, 795*(2), 239–250.

Zhang, L. Y., Tang, X. C., & Sun, M. X. (2005). Simultaneous determination of histamine and polyamines by capillary zone electrophoresis with 4-fluor-7-nitro-2, 1, 3-benzoxadiazole derivatization and fluorescence detection. *Journal of Chromatography B – Analytical Technologies in the Biomedical and Life Sciences, 820*(2), 211–219.

Zotou, A., Loukou, Z., Soufleros, E., & Stratis, I. (2003). Determination of biogenic amines in wines and beers by high performance liquid chromatography with pre-column dansylation and ultraviolet detection. *Chromatographia, 57*(7–8), 429–439.